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Test of Various Insecticides as Systemics in Wheat Upon the Army Cutworm

Lavarre Delancy Uhlken

TEST OF VARIOUS INSECTICIDES AS SYSTEMICS IN WHEAT UPON THE ARMY CUTWORM

BY

LAVARRE DELANCY UHLKEN

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TEST OF VARIOUS INSECTICIDES AS SYSTEMICS IN WHEAT UPON THE ARMY CUTWORM

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the tesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor — Date

Head, Entomology-Zoology Department — Date

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INTRODUCTION

There are many species of cutworms. They vary greatly in population from year to year. The army cutworm, Euxoa auxiliaris (Grote), is very common from the Rockies east to the Great Plains and is occasionally a severe pest to wheat in these areas. Daniels (1964) found that, normally, army cutworms feed largely on the upper portions of plants. Where large populations of this cutworm are present, plants are consumed to the ground. After consuming nearly all the vegetation in one area, cutworms will migrate by the thousands to adjacent fields.

The army cutworm has 1 generation a year, wintering as half-grown larvae. Larvae of the army cutworm rarely feed during warm, sunny days but burrow into the soil and remain quiescent (Strickland 1916). They become active and feed in the late afternoon, at night, and on cool, cloudy days. Day to day feeding is not rhythmic nor continuous but is dependent upon favorable soil and air temperatures.

Endrin, endosulfan and dieldrin have been recommended for the control of the army cutworm, Euxoa auxiliaris (Grote) (Burkhardt 1954, Depew 1959, 1965, Pfadt 1955, 1960). Two methods of insecticide application have been used - broadcast spraying of soil and plant or seed treatment.

This study was made to determine the extent of systemic capability of 2 organic phosphate and 3 carbamate insecticides in the winter wheat plant, Triticum aestivum L. em. Thell. (variety Trapper), grown under greenhouse conditions, the systemic insecticidal effect on feeding fourth and fifth-instar larvae of the army cutworm, and the effect of insecticide soil treatments on the germination of wheat plants.

LITERATURE REVIEW

Depew (1965) conducted field tests in western Kansas to evaluate the effectiveness of various insecticides for the control of the army cutworm infesting winter wheat. Endosulfan at 0.5, endrin at 0.2, and dieldrin at 0.375 pounds active ingredient per acre were more effective than other materials tested. The chemicals tested were sprayed directly upon the wheat - no note of systemic activity was published. Burkhardt (1954) reported that endrin at 0.2 pounds active ingredient per acre was the best insecticide of those tested in alfalfa for control of the army cutworm. McDonald and Jacobson (1958) tested a number of chlorinated hydrocarbon insecticides as contact and stomach poisons on various larval instars of the army cutworm. Endrin was the most toxic of the insecticides tested to sixth-instar larvae and was equally effective either as a contact or as a stomach poison. Fourth and fifth-instar larvae were more readily killed by contact than sixth-instar larvae with each of the compounds tested.

The role of soil type in determining the systemic efficiency of an insecticide has been investigated (Abdellatif et al. 1967, Harris 1966). The physical processes of diffusion and waterflow are necessary to bring about contact between the root system and the soil insecticides. The major problem with soil applications of insecticides in established plantings is to get toxicants from the point of application to the root system. At the root-soil interface, the toxicants can be absorbed and translocated into the plants in quantities sufficient to be toxic to attacking pests without inducing adverse plant effects. Abdellatif et al. (1967) tested various soil types versus systemic efficiency of aldicarb and carbofuran and found that clay content drastically affected the rate of uptake of both insecticides.

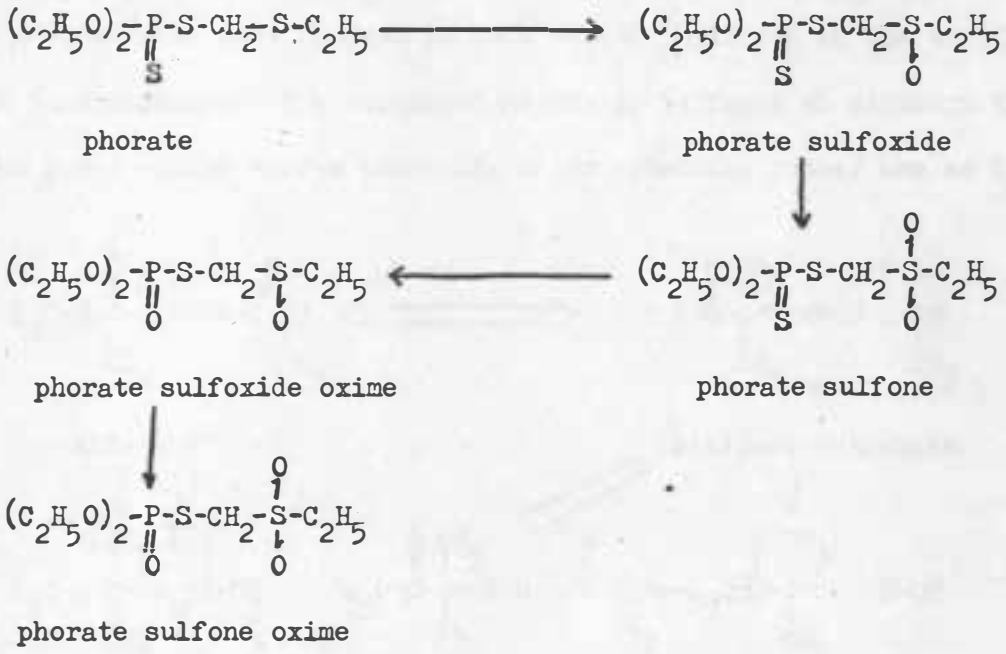
They found that systemic insecticides were absorbed by the roots of cotton plants faster from light soil than from heavy soil. They concluded that organic-matter content plays an important role in the enhanced absorption of systemic insecticides by plants.

The systemic activity of phorate (applied as a seed dressing) in wheat plants was studied by using the aphid, Rhopalosiphum padi (L.), as a test insect (Bardner 1964). Wheat plants quickly lost their toxicity to insects when the plants were transplanted from treated to untreated soil, suggesting that most of the insecticide from a seed treatment passes into the soil and is picked up by the roots. Young and old leaves of wheat depend on continued absorption of phorate from the soil to maintain toxicity. No insecticide moves from old to young leaves. Roots of wheat from treated seed do not excrete insecticide and the roots do not carry insecticide through the soil.

It has been reported that soil microorganisms absorb and metabolize insecticides obtained from the soil (Cosgrove 1967). Amhed and Casida (1958) studied microorganisms and their effect on phorate. The yeast, Torulopsis utilis, the bacteria, Pseudomonas fluorescens and Thiobacillus thiooxidans, and the green alga, Chlorella pyrenoidosa, were used as test organisms. It appeared as if phorate were rapidly absorbed by the above organisms except Chlorella, and then slowly released from the living and dead cells in the cultures as phorate. Chlorella oxidized phorate to phorate sulfoxide which was very stable to hydrolysis.

Metabolism of insecticides has been widely studied in recent years especially the organic phosphate and carbamate insecticides. Metcalf and

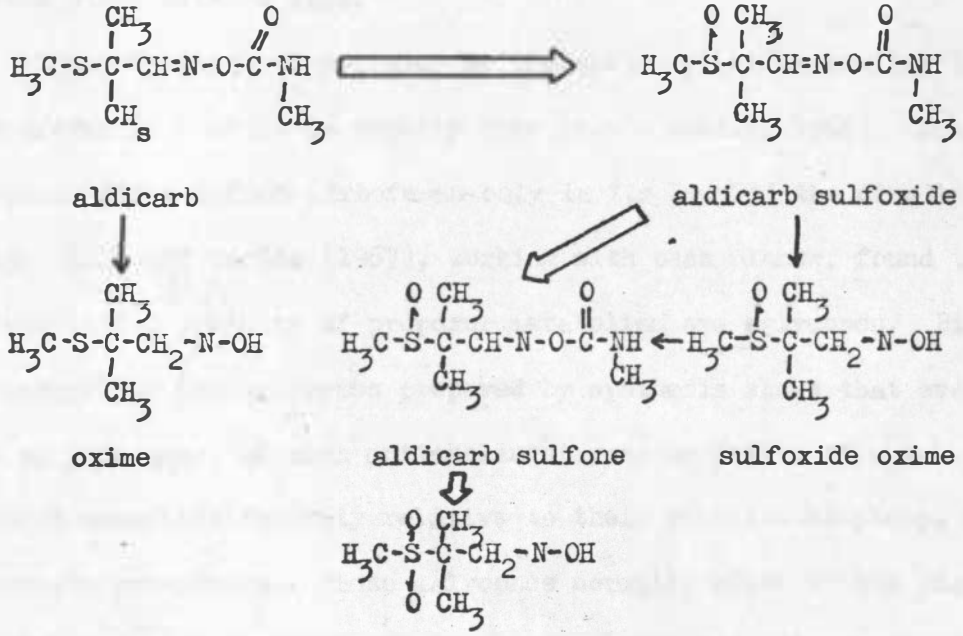
Fukuto (1957) worked out the metabolism of phorate in plants. They found that the predominate processes of plant metabolism for phorate were oxidative. The major pathway of phorate metabolism in plants is shown below.



Getzen and Chapman (1960) found that soil applications of phorate are partially oxidized, hydrolyzed and bound to the soil. Bull et al. (1964) reported that systemic activity of phorate and Cyolane® in cotton plants continued as an effective control of adult boll weevils for 6 weeks. Cyolane and phorate were not translocated in lethal concentrations from old leaves to young leaves.

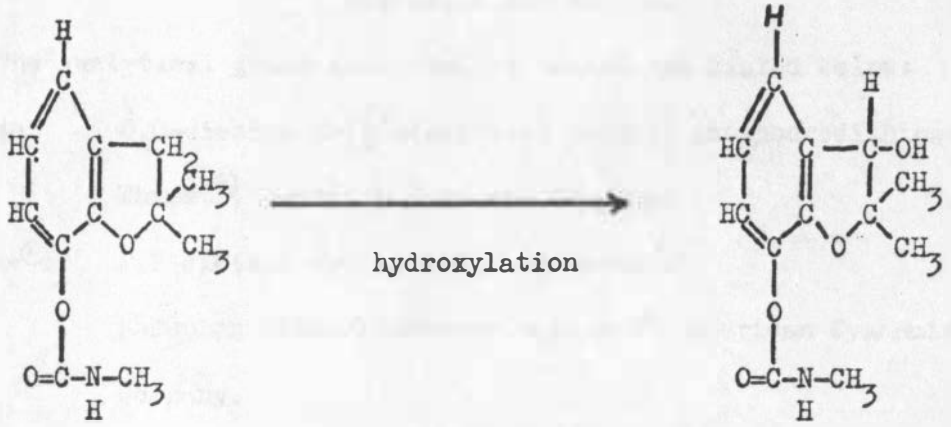
The metabolism of ^{14}C-labelled aldicarb has been investigated in the cotton plant by Metcalf (1966). He found that aldicarb is readily and completely oxidized to its sulfinyl derivative, aldicarb sulfoxide, within 4 to 9 days at moderate temperatures. The sulfoxide, which is more active as a cholinesterases inhibitor, is the active metabolite. The long term

persistence and relatively slow oxidation of the sulfoxide to its sulfonyl derivative, aldicarb sulfone, is responsible for the persistent systemic activity of the insecticide. Aldicarb sulfoxide and aldicarb sulfone are more insecticidal than the parent compound. Metcalf (1966) also demonstrated that phorate is more rapidly oxidized in the wheat plant than aldicarb. The suggested metabolic pathways of aldicarb in the cotton plant (large arrows designate major metabolic route) are as follows:



Carbofuran is metabolized by hydroxylation and hydrolysis in the cotton plant (Metcalf 1968). The key metabolites are produced by hydroxylation at the benzylic carbon to give 3-hydroxy carbofuran which is subsequently oxidized to the 3-keto carbofuran. The 3-keto carbofuran is hydrolytically unstable. Metabolites which were identified with certainty were 3-hydroxy carbofuran, 3-keto carbofuran and their respective 7-hydroxy hydrolysis products. No carbofuran is left in the cotton plant after 8 days.

The metabolism of propoxur by the cotton plant is similar to carbofuran in that it is readily hydrolyzed (Metcalf 1968). Chemically, propoxur differs from carbofuran only in its lack of the dimethyl furan ring. Kuhr and Casida (1967), working with bean plants, found that the hydroxylation products of propoxur metabolism are aglycones. Bioassay of several of the aglycones prepared by synthesis shows that even though the aglycones are of high anticholinesterase activity, they are of reduced mammalian toxicity relative to their substituted-phenyl methyl-carbamate precursors. These aglycones normally exist in the plant as glycosides which probably are less active than the aglycones as cholinesterase inhibitors. Following is a general metabolic pathway of carbofuran in cotton plants as devised by Metcalf (1968).

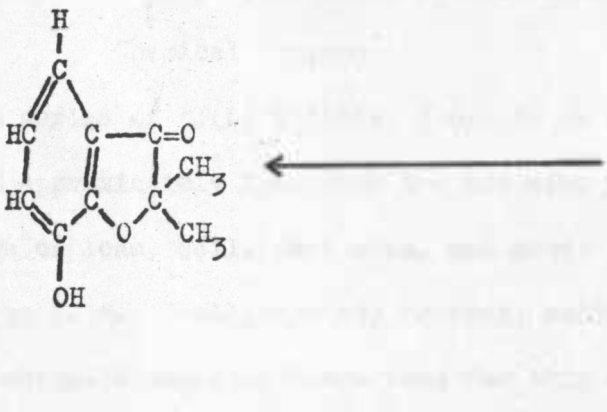


carbofuran

hydroxylation

3-hydroxycarbofuran

oxidation



3-keto carbofuran phenol

3-keto carbofuran

MATERIALS AND METHODS

The analytical grade insecticides tested are listed below:

phorate: O,O-diethyl S- [(ethylthio) methyl] phosphorodithioate, Thimet®, American Cyanamid Company.

Cyolane®: P,P-diethyl cyclic ethylene ester of phosphonodithioimido=carbonic acid, American Cyanamid Company.

aldicarb: 2-methyl-2-methylthio proprionaldehyde -O-methylcarbamoyl oxime, Temik®, Union Carbide Company.

carbofuran: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate, Furadan®, Niagara Chemical Company.

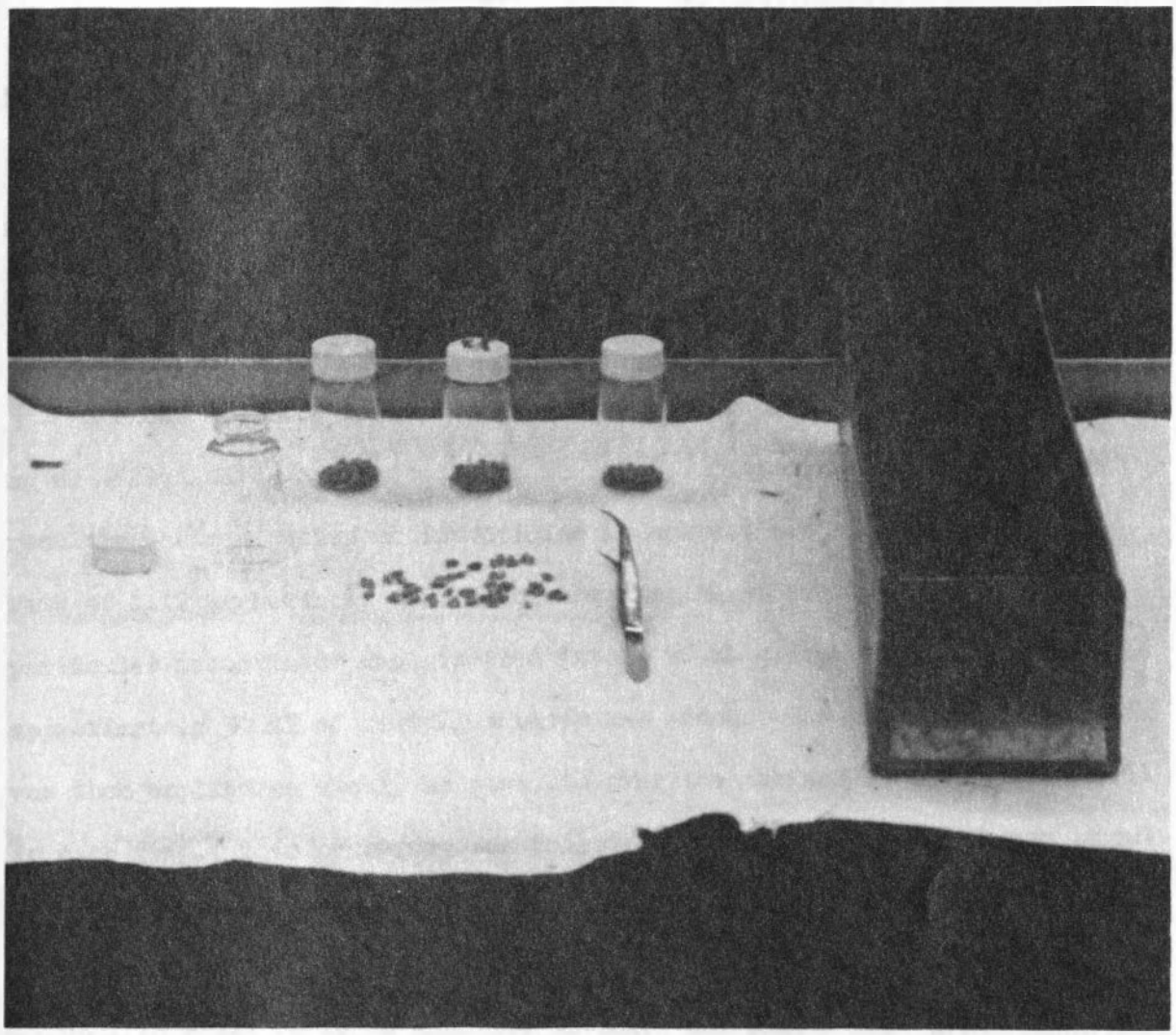
propoxur: O-isopropoxyphenyl methylcarbamate, Baygon®, Chemagro Chemical Company.

A series of 33(L) X 7.6(W) X 6.4(H) cm galvanized steel trays were filled approximately 1½cm from the top with greenhouse potting soil, a mixture of loamy soil, peat moss, and sand. Winter wheat (Triticum aestivum L. em. Thell, variety Trapper) seeds that had no previous history of insecticide treatment were used for this study. Twenty-five selected wheat seeds per tray were planted at a depth of 1.27 cm (½ in). Seeds were planted prior to insecticide treatment of the soil (Figure 1). The insecticides were applied the same day.

Each insecticide was applied to the soil at four rates, 1.12 kg/hectare, 2.24 kg/hectare, 5.60 kg/hectare and 11.2 kg/hectare. Each rate for each insecticide was replicated 5 times.

Since very minute amounts of insecticides were used in this study,

Figure 1.-Winter wheat seeds, Triticum aestivum L. em. Thell. (variety Trapper), galvanized steel tray with greenhouse potting soil.



dilutions had to be made from a stock solution for each insecticide. The amount of insecticide needed for each rate was computed as follows. The area of each tray was 250.8 cm^2, and 1 hectare is equivalent to 1.00 X 10^8 cm^2, so:

$$250.8\ cm^2 \times \frac{1\ \text{hectare}}{1.00 \times 10^8\ cm^2} = 2.51 \times 10^{-6}\ \text{hectares}$$

Each tray represented 2.51 X 10^{-6} hectares. To determine the amount of insecticides needed to be equivalent to each specified rate, the following formula was used:

rate X size of tray = weight of insecticide to be applied, so:

1.12 kg/hectare X 2.51 X 10^{-6} hectare = 2.81 X 10^{-6} kg or 2.81 mg

A stock solution of each insecticide was prepared by dissolving 100 mg of analytical grade insecticide in 100 ml of Nanograde® acetone. The resulting concentration of insecticide in solvent was 1 mg/ml. For the rate of 1.12 kg/hectare, 2.81 ml of the 1 mg/ml stock solution of each particular insecticide was pipetted into a 50 ml graduated cylinder and approximately 50 ml of distilled water was added. The resulting solution was then applied as evenly as possible over the surface of the planted soil in each tray. This procedure was followed for all rates applied throughout the study (Figure 2).

The planted and treated trays were placed in a growing chamber held at 75 $\pm$ 10°F and relative humidity 60-70%. The plants were grown under continuous light, each tray approximately 31 cm directly beneath a fluorescent light source. Each tray was watered daily with distilled water throughout the test period (Figure 3).

Figure 2.-Insecticide application, showing tray, and insecticide stoc
solution (1 mg/ml).

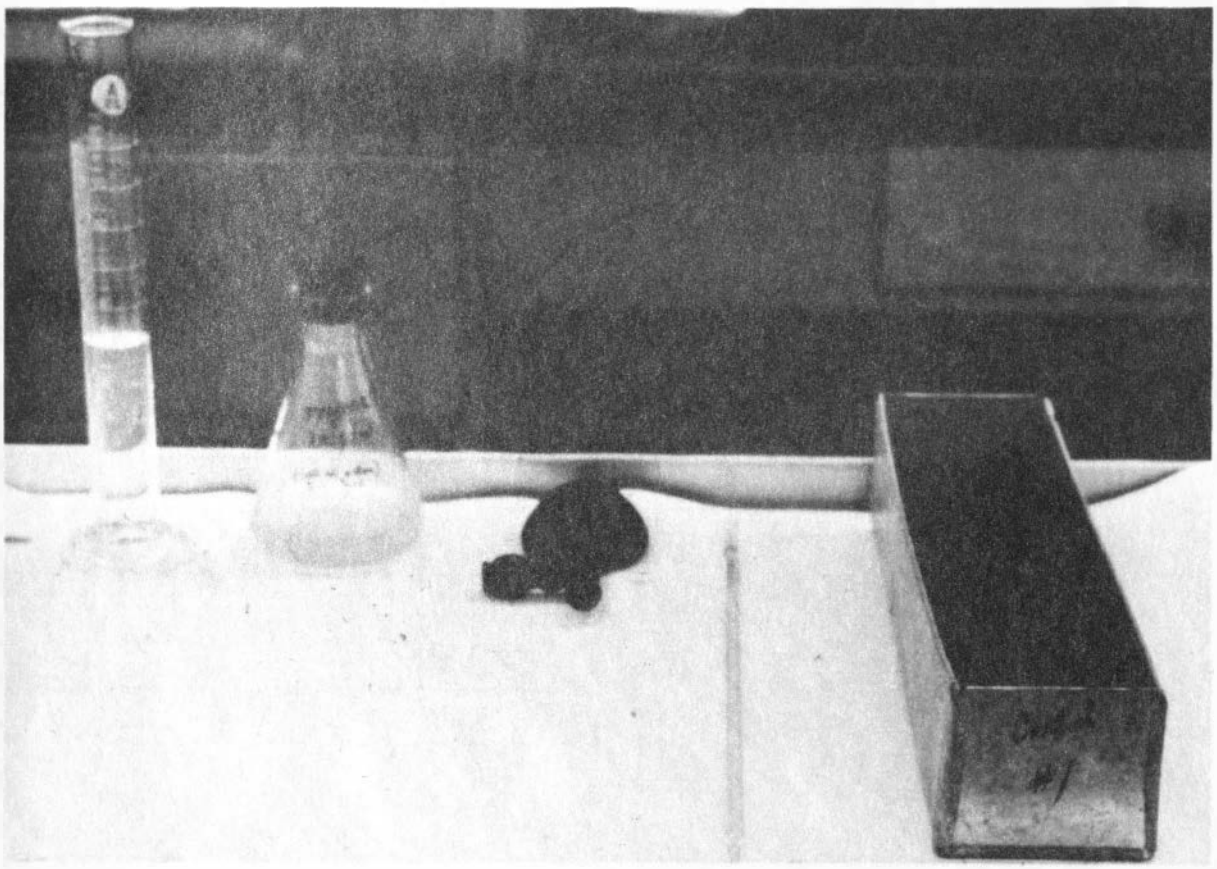
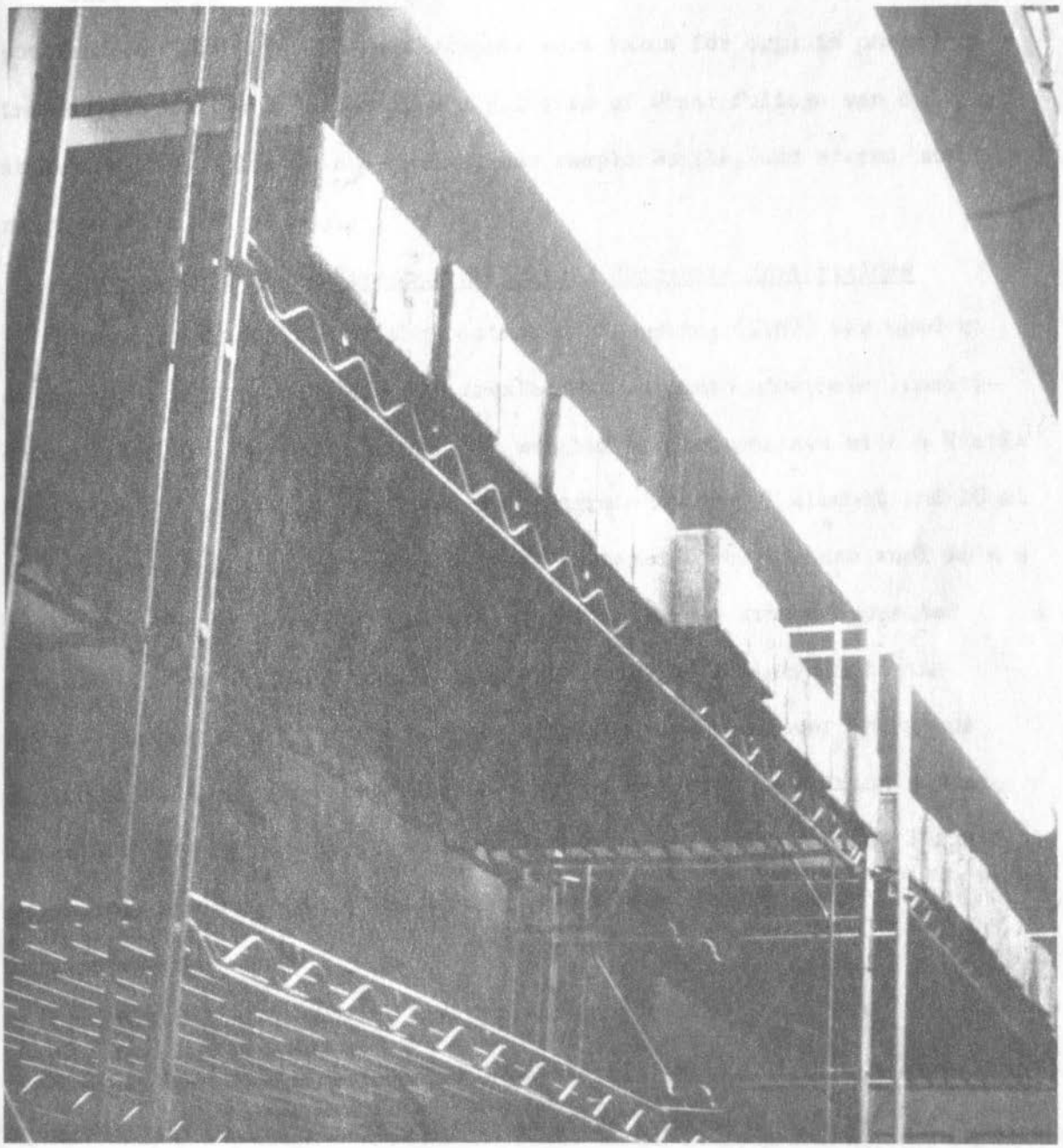


Figure 3.-Artificial growing chamber showing arrangement of trays.



Sampling Procedures

Wheat samples for carbamate insecticide analysis were cut 2 weeks posttreatment. Two and 3-week samples were taken for organic phosphate insecticide analysis. Approximately 1 gram of wheat foliage was cut just above the soil, placed in a small glass sample bottle, and stored under refrigeration (Figure 4).

Extraction and Purification of Organic Phosphate Insecticides

The following mixed solvent method of Thornberg (1963) was used to extract the winter wheat samples treated with organic phosphate insecticides. One gram of wheat sample was weighed and homogenized with a Virtis "45"® blendor (Figure 5) in 20 ml of Nanograde isopropyl alcohol and 10 ml of Nanograde benzene. Homogenates were filtered through glass wool into a separatory funnel, and, by a series of partitionings with a saturated aqueous salt solution, the insecticide present was isolated into the upper benzene fraction. The benzene fraction was dried over anhydrous sodium sulfate and pipetted into a graduated cylinder containing nuchar attaclay. The latter mixture was filtered through a Whatman No. 1 filter paper into a glass bottle and the benzene eluate stored under refrigeration.

Purification of phorate and Cyolane samples used a column chromatographic system devised by Langlois et al. (1963). To a 20 mm Pyrex® liquid chromatograph column was added 5 grams of florisil (60-100 mesh). This was washed with 25 ml of 1:1 methylene chloride: petroleum ether. Five grams of florisil (60-200 mesh) was added to the benzene - insecticide residue and mixed under partial vacuum until dry. This florisil mixture was added to the top of the column and eluted with

Figure 4.-Sampling of insecticide residues in wheat showing 1 gram sa
and sample extract.

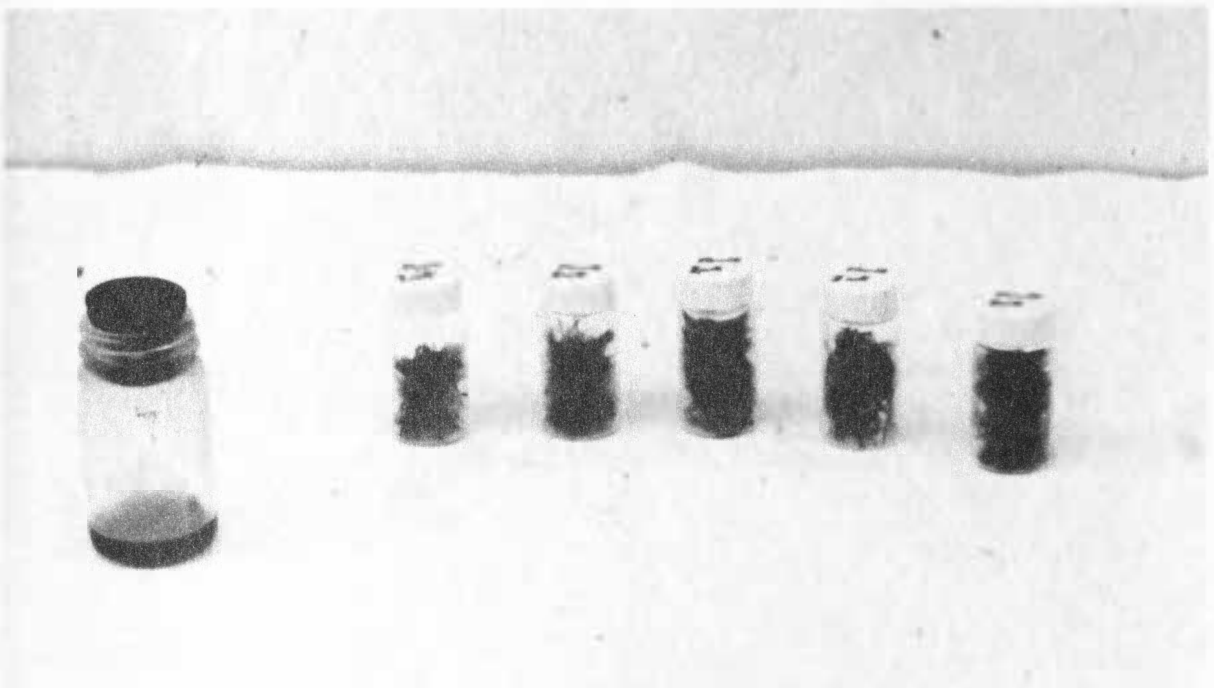
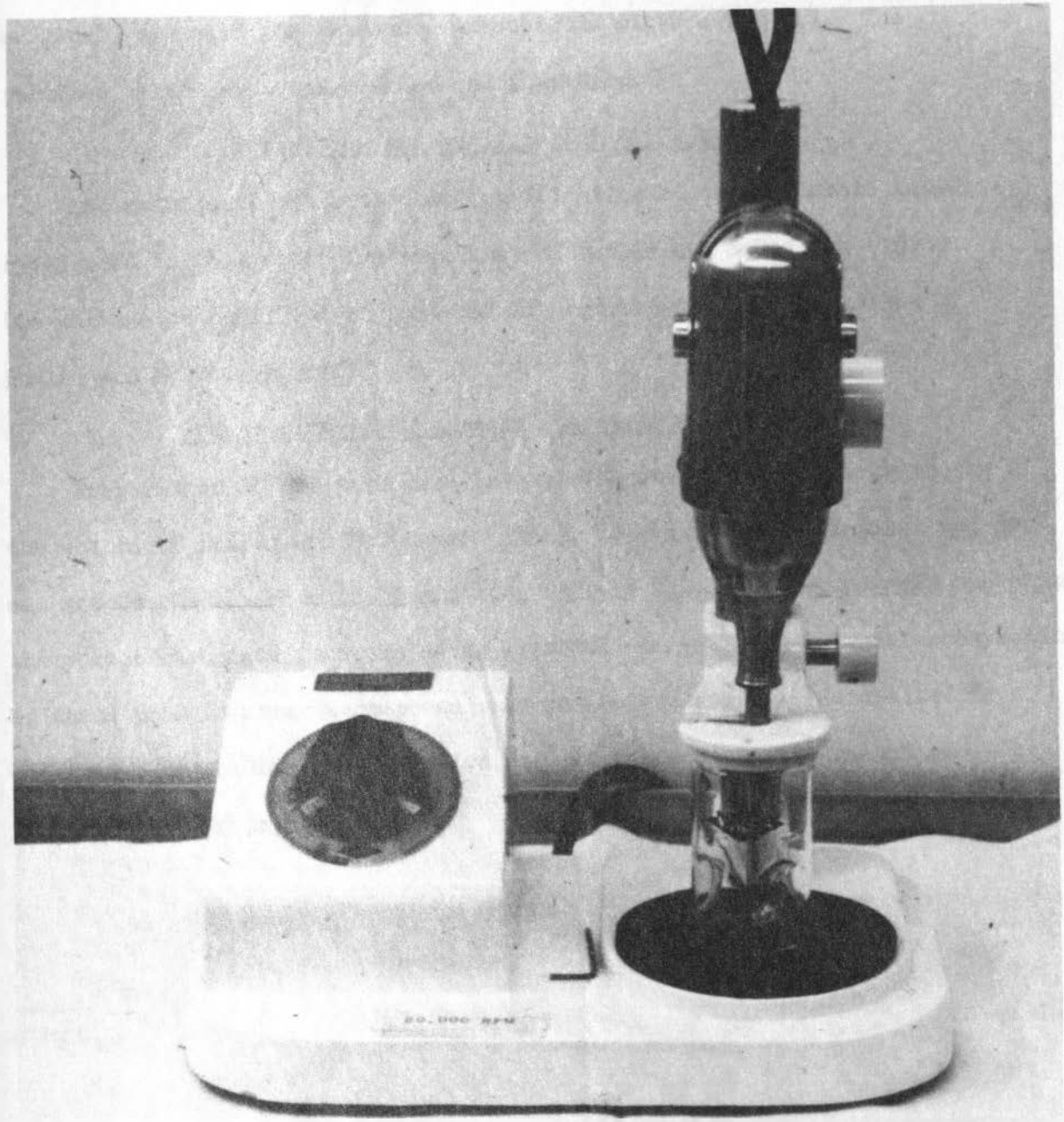


Figure 5.-Virtis "45"® blendor used to homogenize wheat samples.



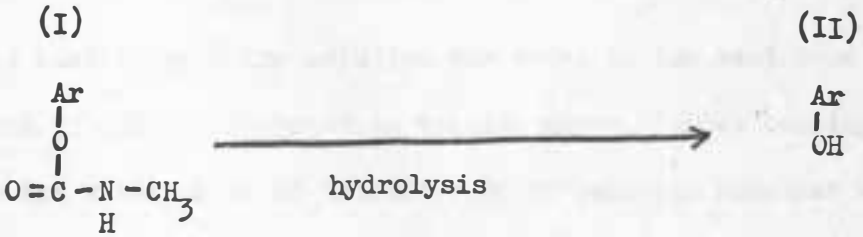
125 ml of 1:4 methylene chloride:petroleum ether. The eluted mixture was evaporated to dryness under partial vacuum and reconstituted to the original volume of the benzene - insecticide mixture (10 ml). The purified sample was stored under refrigeration.

Extraction and Purification of Carbamates

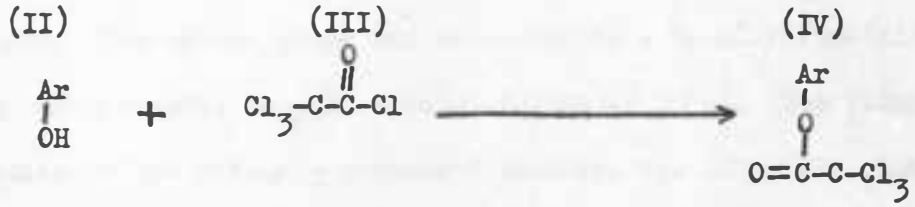
The methods of extraction and purification of the carbamate insecticides used in this study followed that of Butler and McDonough (1968). The carbamates could not be successfully extracted using the method of Butler and McDonough (1968).

Preparations of Carbamate Insecticide Standards

Preparation of the carbamate insecticide standards was according to the method of Butler and McDonough (1968). Carbamate insecticides, per se, can not be easily detected by electron capture GLC. The method used hydrolyzes the parent carbamate insecticide to a phenol derivative. Trichloroacetylation of these phenols forms a compound that can be successfully determined by electron capture GLC. The equation for these reactions is summerized on the following page.



parent carbamate phenol derivative



phenol derivative trichloroacetyl chloride Chlorinated derivative

Product IV is that compound which is detectable by electron capture GLC. An electron capture detector is very sensitive to chlorine atoms that may be present in a compound, and, as shown above, chlorine atoms are present in the carbamate derivative.

One hundred mg of analytical grade carbamate insecticide were dissolved in 10 ml of chloroform. The solution was diluted 1:10 and then 1:2 with chloroform to make a 500 ng/ul solution. One ml of the 500 ng/ul insecticide stock solution was pipetted into a test tube, 0.1 ml of mineral oil solution added, and the contents of the test tube were evaporated to dryness with a gentle stream of air in a 40°C water bath. All subsequent evaporations were carried out in this manner. A 0.2 ml aliquot of a 0.1N sodium hydroxide in methanol solution was added to the test tube, the test tube rotated to wet its sides, and the methanol evaporated. One ml of

spectral quality pyridine solution was added to the test tube and the methylene chloride evaporated in boiling water. After cooling the test tube in ice water, 1 ml of trichloroacetyl chloride solution was added and the methylene chloride evaporated. A number of partitionings were made using distilled water, a saturated sodium bicarbonate solution and Nanograde hexane. The desired phenolic derivative partitioned into the hexane phase. The hexane phase was collected in a 50 ml volumetric flask and enough hexane added to make a total volume of 50 ml. The final concentration of the hexane - carbamate standard was 10 ng/ul. The prepared reference standard was stored under refrigeration.

Percent Recovery of Organic Phosphate Insecticides

A 1 ml aliquot of a 100 ng/ul standard solution of phorate (500 ng/ul for Cyolane) in benzene was pipetted into a Virtis "45" blendor. One gram of untreated wheat foliage was weighed and placed into the blendor along with a 1:2 mixture of benzene(10 ml):isopropyl alcohol(20 ml). The extraction method of Thornberg (1963) was followed. Purification of the fortified wheat sample was done according to the method of Langlois et al. (1963). The purified eluate was reconstituted to 10 ml of benzene to make a 10 ng/ul solution of phorate (50 ng/ul solution of Cyolane). The fortified samples were analyzed by GLC accompanied by an appropriate standard.

Gas-Liquid Chromatography Methods

Gas-liquid chromatography (Varian Aerograph® model 600-C, Figure 6, with electron capture tritium detector) was used to determine the insecticides in the wheat samples. Phorate and Cyolane were separated on a 183 cm X 0.317 cm glass column packed with Chromosorb W coated with

Figure 6.-Varian Aerograph® model 600-C used to determine insecticide residues in wheat foliage.


CHART SPEED
Hy-Fi
RANGE
ATTENUATOR
POWER

10% Dow 11. Assay of carbamate insecticides was made by using a 152 cm X 0.317 cm stainless stell column packed with Varaport 70/80 coated with 10% DC 200. The operating parameters for determination of both organic phosphate and carbamate insecticides were: oven temperature 190°C, injector temperature 200°C, voltage EC-10, range EC-1, attenuation 1x, nitrogen carrier flow rate of 50 ml/min, recording chart speed of 1.27 cm/min (Sargent® recorder model SR), sample injection size 1 ul.

Sample peak areas recorded from GLC were calculated by triangulation. Quantitative results were obtained by comparison of sample peak areas with peak areas of known concentrations of standards.

Calibration curves were made for phorate and Cyolane in order to determine the linear dynamic range of the tritium detector for these 2 insecticides. A series of 1 ul injections of an increased concentration of insecticide was plotted against the areas of the appropriate peaks using log-log paper (Figures 7 and 8).

Residue Determination

Residues of phorate and Cyolane in 2 and 3-week old wheat foliage were determined as follows. Standards of phorate and Cyolane were prepared by dissolving 100 mg of analytical grade insecticide in Nanograde benzene and diluting to a concentration that was within the linear dynamic range of the tritium detector. By referring to the linearity curves of phorate and Cyolane (Figures 7 and 8), one could select a concentration somewhere near the midpoint of the linear dynamic range. A series of 1 ul injections of a known concentration of insecticide were made before the sample extracts were injected (it is advisable to inject standards immediately before making the sample extract injections because sen-

Figure 7.-Linearity curve of phorate. (Concentration plotted against in mm^2 of peak).

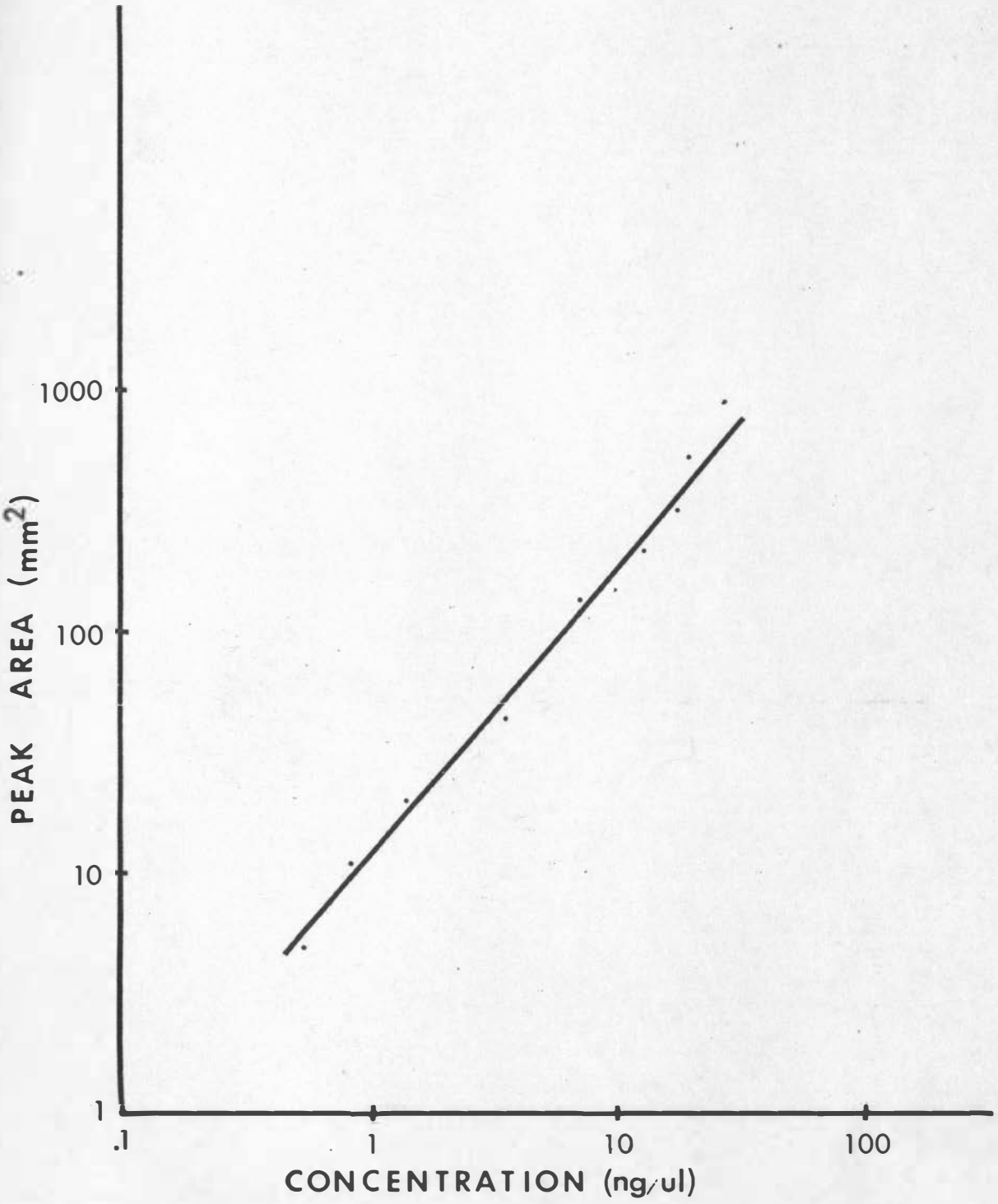
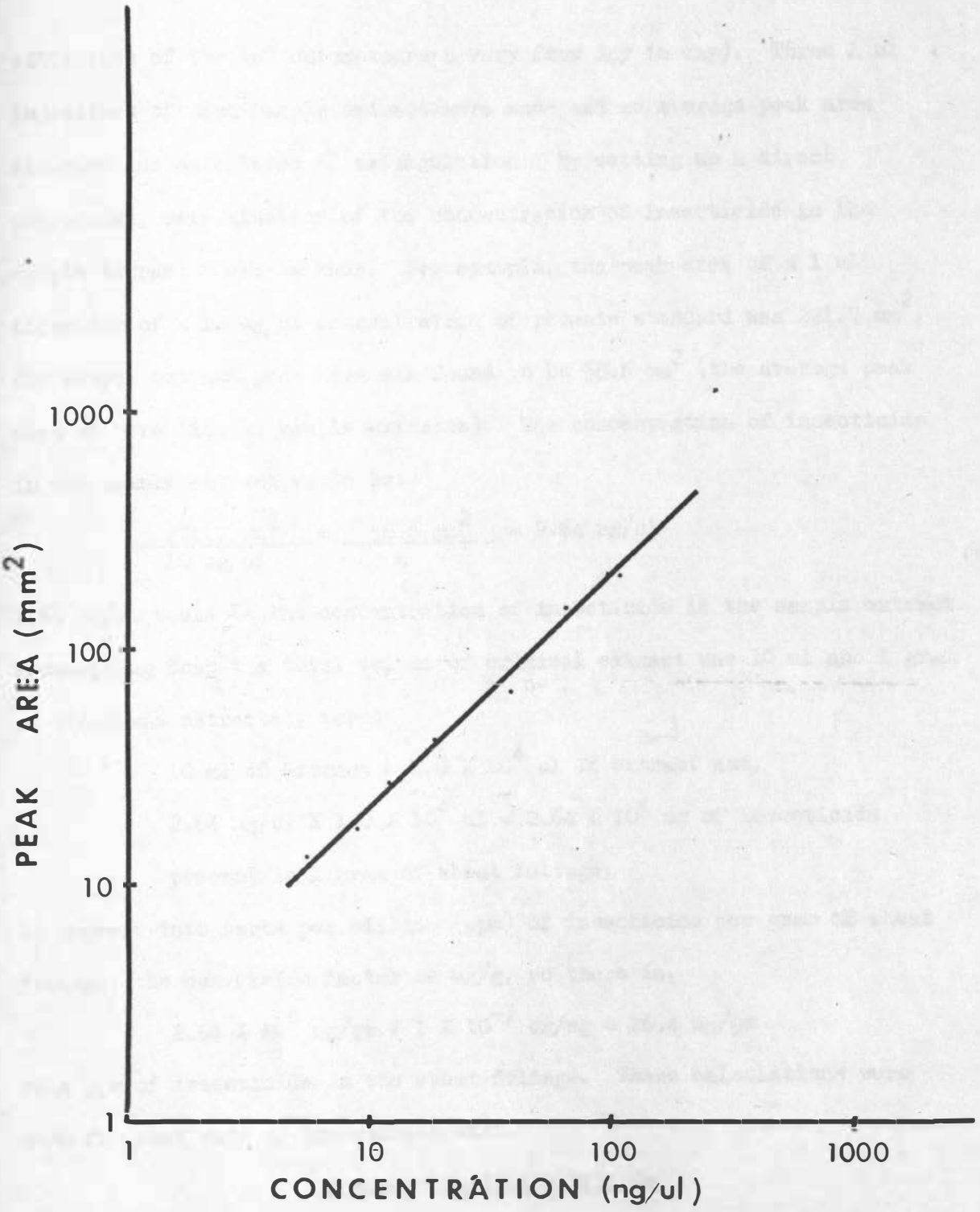

1000
100
10
1
PEAK AREA (mm^2)
.1
1
10
100
CONCENTRATION (ng/ul)

Figure 8.-Linearity curve of Cyolane®. (Concentration plotted against area in mm^2 of peak).


1000
100
10
1
PEAK AREA (mm^2)
1
10
100
1000
CONCENTRATION (ng/ul)

sitivities of the gas chromatograph vary from day to day). Three 1 ul injections of each sample extract were made and an average peak area recorded and calculated by triangulation. By setting up a direct proportion, determination of the concentration of insecticide in the sample extract could be made. For example, the peak area of a 1 ul injection of a 10 ng/ul concentration of phorate standard was 221.7 mm^2. The sample extract peak area was found to be 58.6 mm^2 (the average peak area of 5 replicated sample extracts). The concentration of insecticide in the sample extract would be:

$$\frac{221.7\ mm^2}{10\ ng/ul} = \frac{58.6\ mm^2}{x} = 2.64\ ng/ul$$

2.64 ng/ul would be the concentration of insecticide in the sample extract. Remembering that the total volume of original extract was 10 ml and 1 gram of wheat was extracted, then:

10 ml of extract = 1.0×10^4 ul of extract and,

$2.64\ ng/ul \times 1.0 \times 10^4\ ul = 2.64 \times 10^4$ ng of insecticide present in 1 gram of wheat foliage.

To convert into parts per million (ppm) of insecticide per gram of wheat foliage, the conversion factor is ug/g, so there is,

$$2.64 \times 10^4\ ng/gm \times 1 \times 10^{-3}\ ug/ng = 26.4\ ug/gm$$

26.4 ppm of insecticide in the wheat foliage. These calculations were made for each rate of insecticide used.

Dosage - Mortality Methods

A series of 7 concentrations (0.01, 0.05, 0.10, 0.50, 1.00, 5.00, 10.00 ug/ul) for each test insecticide, was applied directly to ½ inch army cutworm larvae. Three replicates of 10 larvae each were topically

treated with 1 ul of a diluted insecticide solution delivered by a microapplicator for each concentration in the series. At 24 hours posttreatment the number of affected cutworms was determined and recorded. The larvae were exposed to elevated temperature and if no disorganized movement was noted or if larvae did not react, these larvae were counted as being affected.

Bioassay of Plant Insecticides

A bioassay to determine the insecticidal content of treated wheat was made using $\frac{1}{2}$ inch army cutworm larvae. The wheat plants had been treated prior to the tests. Five replicates of 10 larvae each were placed individually in glass petri dishes and the cutworms starved for 15 hours. Approximately 1 gram of 2-week old wheat was placed in plastic petri dishes containing wet Whatman No. 1 filter paper to prevent dessication of the wheat foliage. Ten starved army cutworm larvae were placed in each petri dish containing wheat, covered, and kept in a dark room at room temperature. Larval mortality counts were taken at 6 hour intervals, terminating with a 24-hour count. A probit analysis was used to determine the LD_{50}. An electronic computer was utilized to facilitate speed of accuracy for obtaining LD_{50} values.

Germination of Wheat Plants

The number of wheat plants emerging each day in the test trays was recorded for 8 days (the number emerging was converted to percent of total seeds planted) in order to determine whether or not there was a significant difference between the rate of emergence of wheat plants in treated soil versus untreated soil (control). A generalized analysis of variance with equal subclass numbers was used to determine significant differences.

RESULTS

The carbamate insecticides, *per se*, could not be analyzed by electron capture GLC. A prepared derivative (standard) of each carbamate was necessary for analysis. Carbofuran derivatives could be detected. However, the sensitivity of detection was low (Table 1). Recoveries of aldicarb and propoxur could not be determined because of problems in preparation and analysis of derivatives needed for GLC techniques.

Phorate and Cyolane, *per se*, could be quantitatively determined by electron capture GLC. Phorate recoveries were high (99.2%). Cyolane recoveries (65.5%) were lower than those for phorate.

The levels of phorate in 2-week old wheat plants depended upon the amount of insecticide applied to the soil (Table 2). After 2 weeks posttreatment, 26.4 ppm of phorate were found in wheat foliage grown in soil treated at the lowest rate and 143.2 ppm of phorate was present in wheat foliage grown in soil treated at the highest rate. As the rate of phorate applied to the soil increased, the residues in wheat foliage increased (Table 2). The residues of phorate in wheat foliage increased, but not at a linear rate, as applied insecticide doses increased.

Residues of Cyolane in 2-week old wheat foliage were greater than those of phorate. Two-week old wheat foliage contained 99.1 ppm of Cyolane (1.12 kg/hectare treatment). As the rates of Cyolane application increased from 1.12 kg/hectare, the residues of Cyolane increased at a non linear rate.

Residues of phorate and Cyolane in wheat foliage (soil treated at the equivalent rate of 1.12 kg/hectare) decreased from 26.4 to 13.7 ppm and 99.1 to 38.0 ppm respectively 3 weeks posttreatment (Table 3).

Table 1.-Percent recoveries and detection limit of phorate, Cyolane®, and carbofuran using electron capture gas-liquid chromatography.

	Percent recovery[a/]	Detection limit (nanograms)
phorate	99.2	0.3
Cyolane®	65.5	3.0
carbofuran	25.0	5.0

a/ Percent recovery calculated in the wheat plant only.

Table 2.-Residues of phorate and Cyolane® in 2-week old wheat foliage.

Insecticide	Rate (kg/hectare)	Concentration in extract (ng/ul)	ug of insecticide per gram of wheat (ppm)
Phorate	1.12	2.64	26.4
	2.24	5.15	51.5
	5.60	8.37	83.7
	11.20	14.32	143.2
Cyolane®	1.12	9.91	99.1
	2.24	11.05	110.5
	5.60	20.59	205.9
	11.20	40.16	401.6

Table 3.-Residues of phorate and Cyolane® in the wheat plant grown in soil treated at the rate of 1.12 kg/hectare at 3 weeks posttreatment.

Insecticide	Concentration in extract (ng/ul)	ug of insecticide per gram of wheat (ppm)
phorate	1.37	13.70
Cyolane®	3.80	38.00

The toxicity of the test insecticides to ½ inch army cutworm larvae was determined by dosage-mortality tests. The LD_{50} of topically treated larvae was established by probit analysis of the dosage-mortality data. Carbofuran was the most toxic to the larvae, phorate and Cyolane about equally toxic, and aldicarb and propoxur demonstrated the least toxicity of those insecticides tested (Table 4).

Bioassay indicated that Cyolane was the most effective systemic of those insecticides tested (Tables 5, 6, 7 and 8). Mortalities of cutworm larvae exposed to wheat that had been treated at the equivalent rate of 1.12 kg/hectare were low. Although none of the test insecticides applied at the rate of 1.12 kg/hectare were considered effective as systemics, aldicarb caused the highest mortality. Cyolane was effective as a systemic insecticide at 2.24, 5.60 and 11.20 kg/hectare. The other insecticides (phorate, propoxur, carbofuran and aldicarb) can not be considered systemics on the basis of the results obtained.

A computerized analysis of variance was obtained from the wheat germination data. The analysis of variance indicated no significant differences between replicates versus levels of insecticide application, and no significant difference between treatments versus days. A significant difference was, however, indicated between replicates versus treatments. Duncan's new multiple range test was used to test for significant differences between treatments (Steele and Torrie 1960). Significant differences were determined at the 0.05% level. Phorate and propoxur treated soil caused a significantly higher germination of wheat seeds than the other insecticides tested and the control. Germination of wheat seeds planted in soil treated with carbofuran, Cyolane and aldicarb did not differ significantly from the

Table 4.-Dosage-mortality data: number of army cutworm larvae affected 24 hours posttreatment.

Insecticide	Rep	Control	0.01	0.05	0.10	0.50	1.00	5.00	10.00	Computed LD_{50}
					(ug/ul)					
Cyolane®	1	0	0	1	1	0	2	2	10	1.980
	2	0	0	0	1	1	3	7	10	
	3	0	0	0	1	0	6	8	10	
phorate	1	0	0	1	0	2	2	10	10	1.750
	2	0	0	1	1	0	2	8	10	
	3	1	0	1	0	3	3	10	10	
carbofuran	1	1	0	3	6	6	10	10	10	0.105
	2	0	0	4	7	10	10	10	10	
	3	1	0	3	5	8	9	10	10	
aldicarb	1	0	0	0	1	0	3	6	8	5.018
	2	0	0	0	1	2	0	1	7	
	3	0	0	0	0	2	0	5	8	
propoxur	1	0	1	2	1	0	3	2	9	10.607
	2	0	1	0	0	0	0	3	6	
	3	0	0	0	2	0	2	4	7	

Table 5.-Mortality of $\frac{1}{2}$ inch army cutworm larvae exposed to wheat treated at the rate of 1.12 kg/hectare.

Insecticide	Percent mortality (average of 5 replicates)			
	6 hours	12 hours	18 hours	24 hours
phorate	0	0	2	2
Cyclane®	2	2	2	2
carbofuran	0	0	0	0
propoxur	0	2	2	2
aldicarb	0	4	6	6
control	2	2	4	4

Table 6.-Mortality of $\frac{1}{2}$ inch army cutworm larvae exposed to wheat treated at the rate of 2.24 kg/hectare.

Insecticide	Percent mortality (average of 5 replicates)			
	6 hours	12 hours	18 hours	24 hours
phorate	6	6	6	6
Cyclane®	4	10	10	20
carbofuran	0	0	0	2
propoxur	2	6	8	14
aldicarb	0	0	0	2
control	0	0	2	2

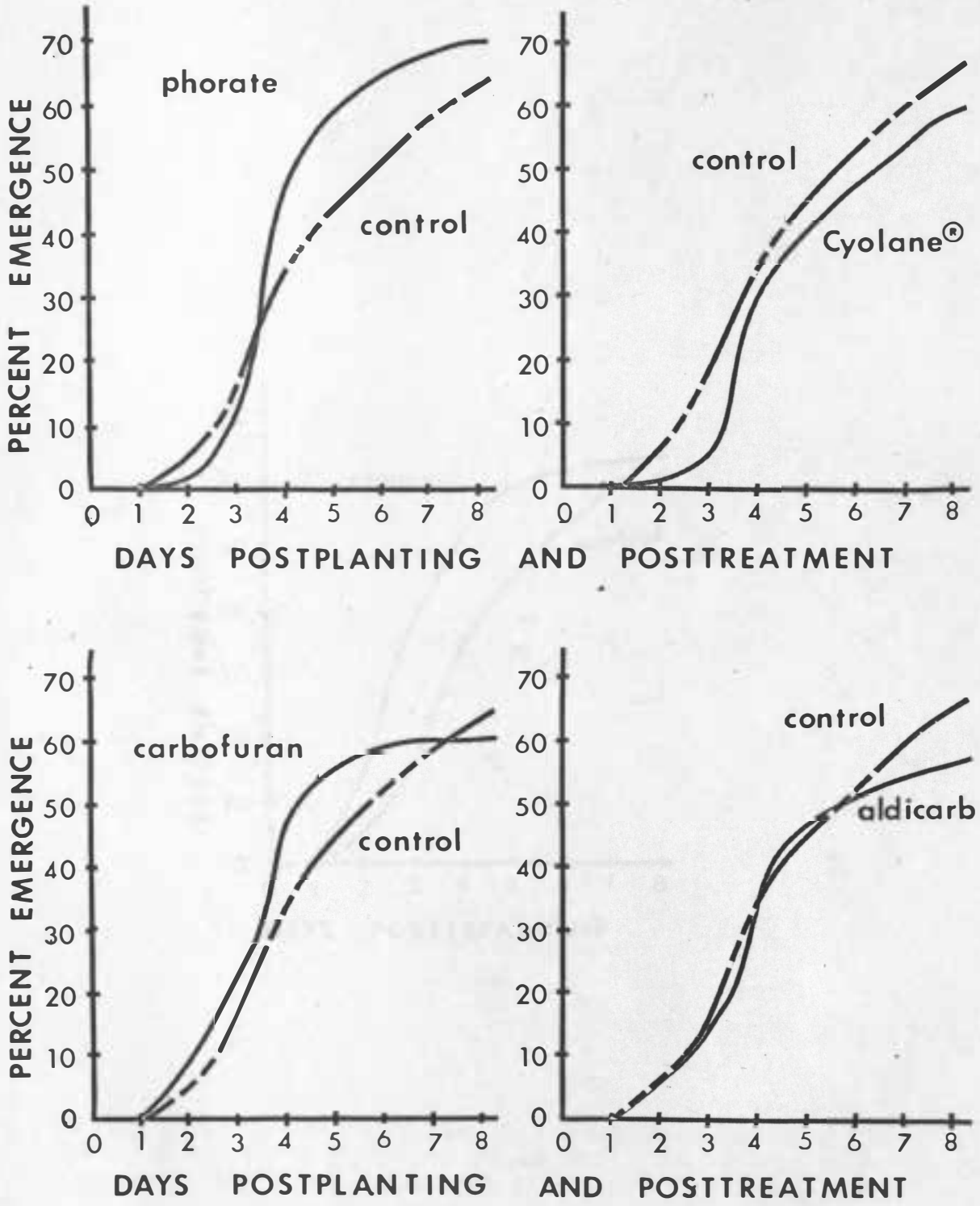
Table 7.-Mortality of $\frac{1}{2}$ inch army cutworm larvae exposed to wheat treated at the rate of 5.60 kg/hectare.

Insecticide	Percent mortality (average of 5 replicates)			
	6 hours	12 hours	18 hours	24 hours
phorate	0	0	0	8
Cyolane®	2	4	18	24
carbofuran	0	0	0	0
propoxur	2	2	2	2
aldicarb	2	2	4	4
control	0	0	0	0

Table 8.-Mortality of ½ inch army cutworm larvae exposed to wheat treated at the rate of 11.20 kg/hectare.

Insecticide	Percent mortality (average of 5 replicates)			
	6 hours	12 hours	18 hours	24 hours
phorate	2	2	2	2
Cyolane®	4	36	64	84
carbofuran	4	6	8	8
propoxur	2	2	2	2
aldicarb	0	0	0	0
control	0	2	4	4

controls. Figures 9, 10, 11 and 12 show germination of wheat seeds planted in soil treated at 1.12, 2.24, 5.60 and 11.20 kg/hectare of insecticide respectively versus the germination of wheat seeds planted in soil not treated (control).


PERCENT EMERGENCE
70
60
50
40
30
20
10
0
phorate
control
0 1 2 3 4 5 6 7 8
DAYS POSTPLANTING AND POSTTREATMENT
control
Cyolane®
carbofuran
control
control
aldicarb

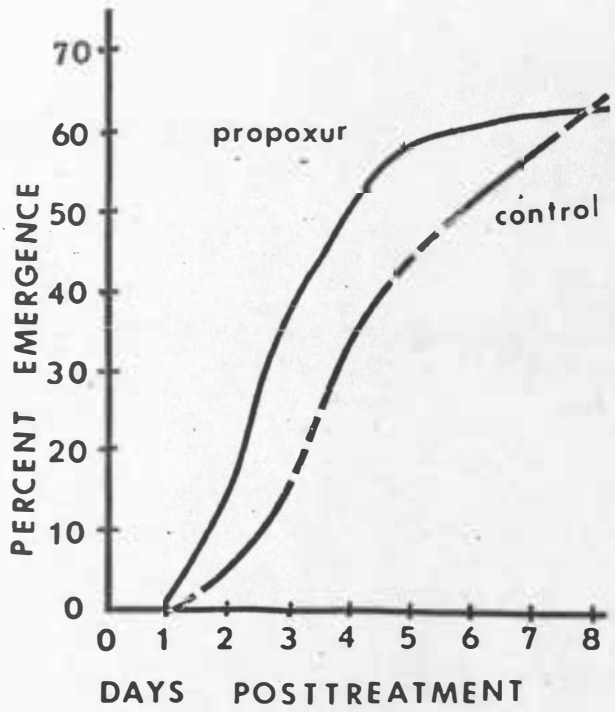
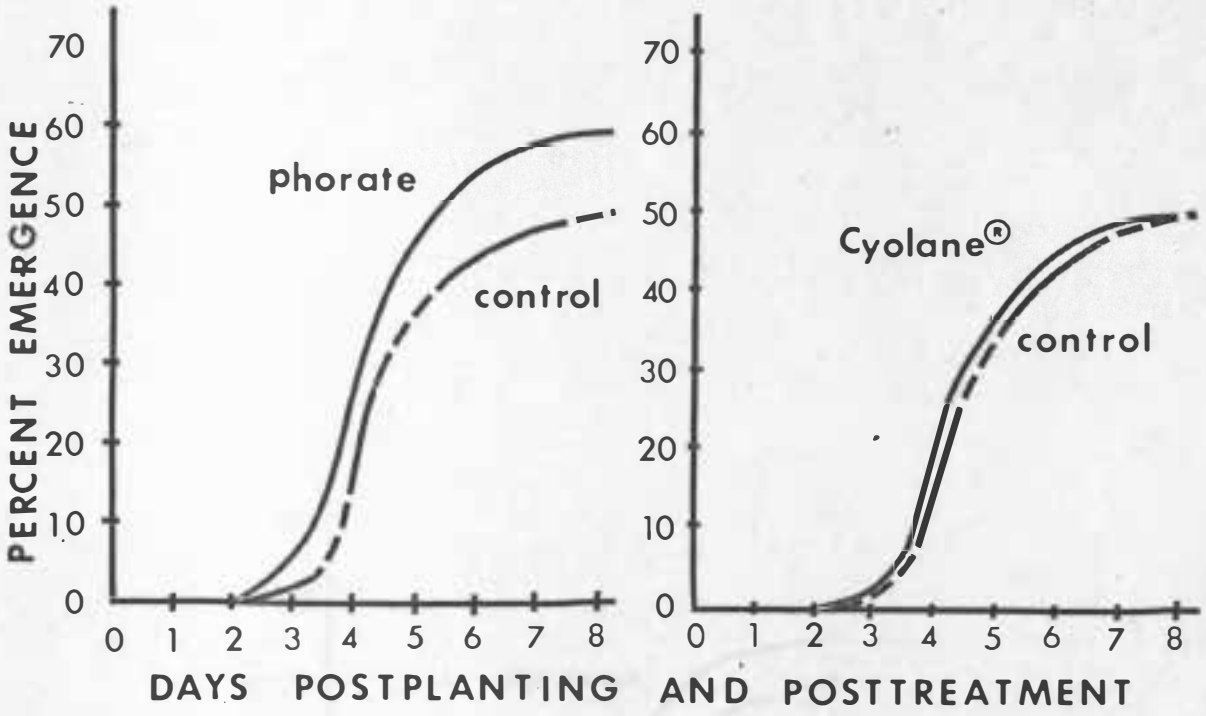
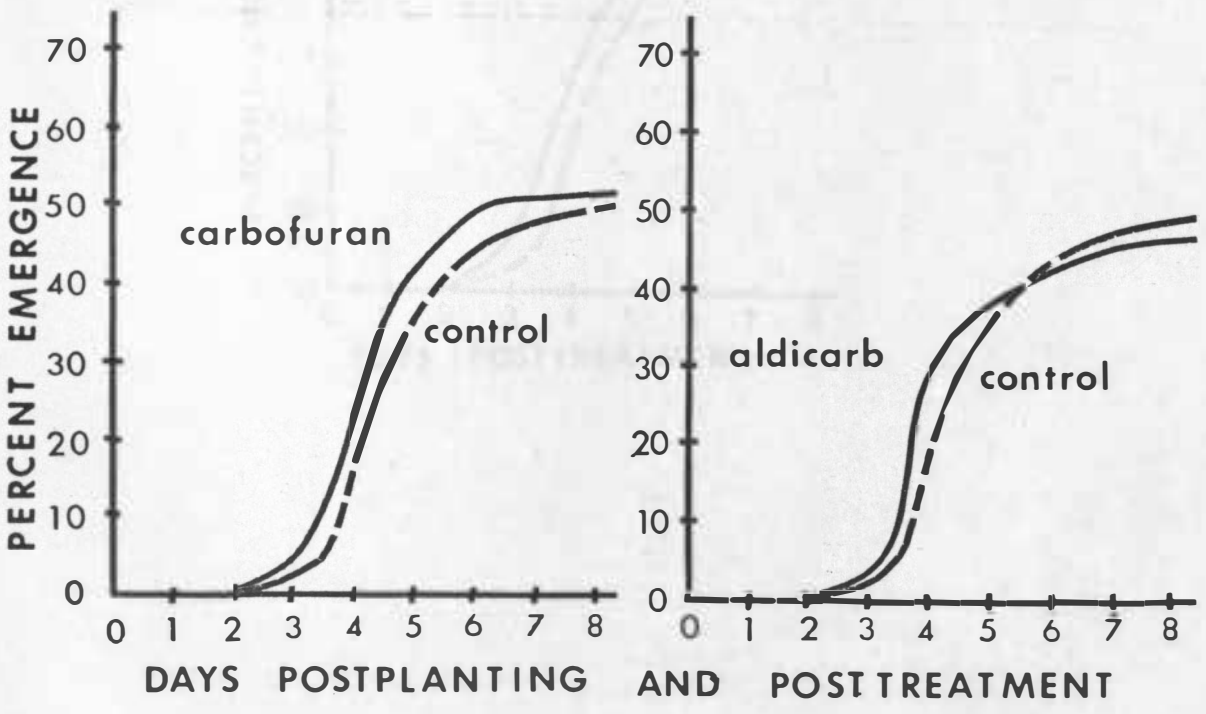

70
60
50
40
30
20
10
0
PERCENT EMERGENCE
propoxur
control
0 1 2 3 4 5 6 7 8
DAYS POSTTREATMENT

Figure 10.-Percent emergence of wheat grown in soil treated with insecticides at the rate of 2.24 kg/hectare.


PERCENT EMERGENCE
70
60
50
40
30
20
10
0
phorate
control
0 1 2 3 4 5 6 7 8
DAYS POSTPLANTING AND POSTTREATMENT
Cyolane®
control


PERCENT EMERGENCE
70
60
50
40
30
20
10
0
carbofuran
control
0 1 2 3 4 5 6 7 8
DAYS POSTPLANTING AND POST TREATMENT
aldicarb
control

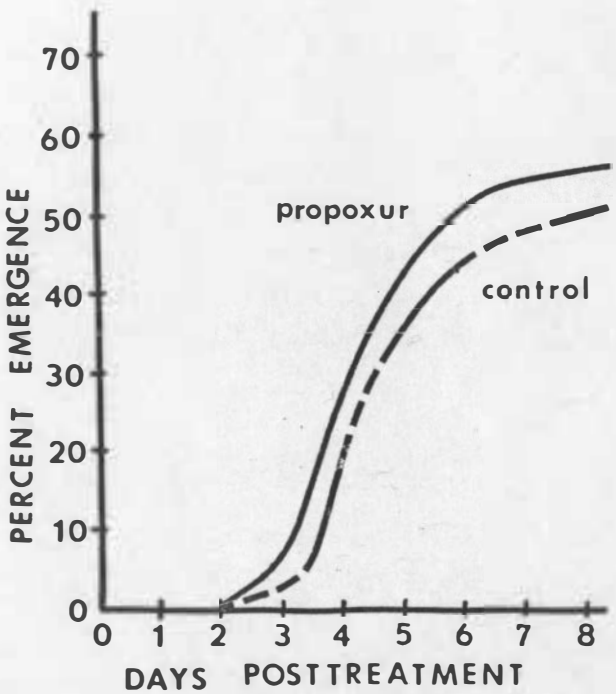
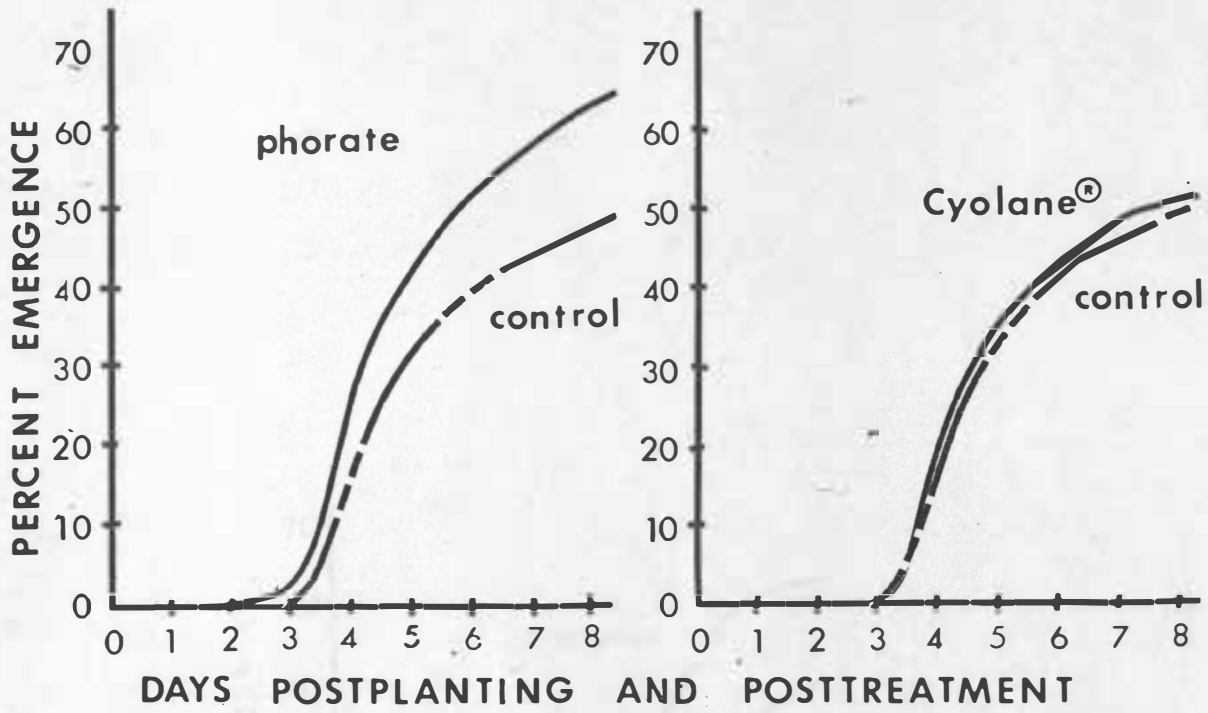
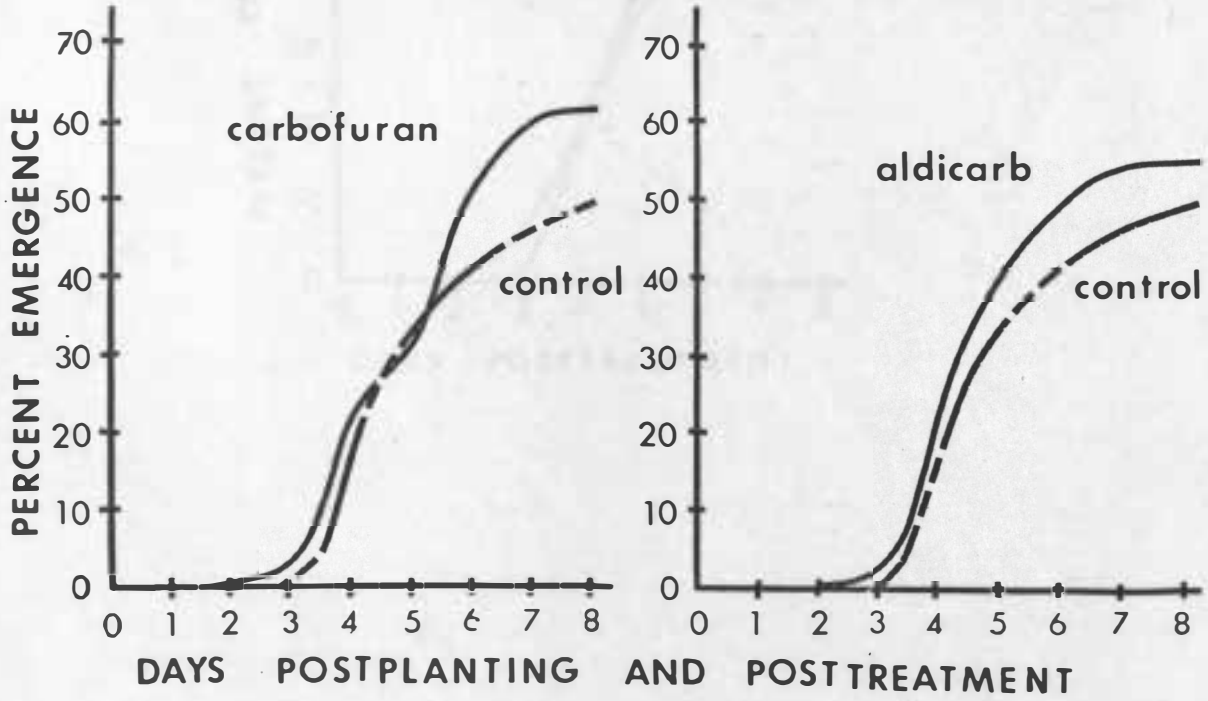

70
60
50
40
30
20
10
0
PERCENT EMERGENCE
propoxur
control
0 1 2 3 4 5 6 7 8
DAYS POSTTREATMENT

Figure 11.-Percent emergence of wheat grown in soil treated with insecticides at the rate of 5.60 kg/hectare.


PERCENT EMERGENCE
70
60
50
40
30
20
10
0
phorate
control
Cyolane®
control
0 1 2 3 4 5 6 7 8
DAYS POSTPLANTING AND POSTTREATMENT


PERCENT EMERGENCE
70
60
50
40
30
20
10
0
carbofuran
control
aldicarb
control
0 1 2 3 4 5 6 7 8
DAYS POSTPLANTING AND POSTTREATMENT

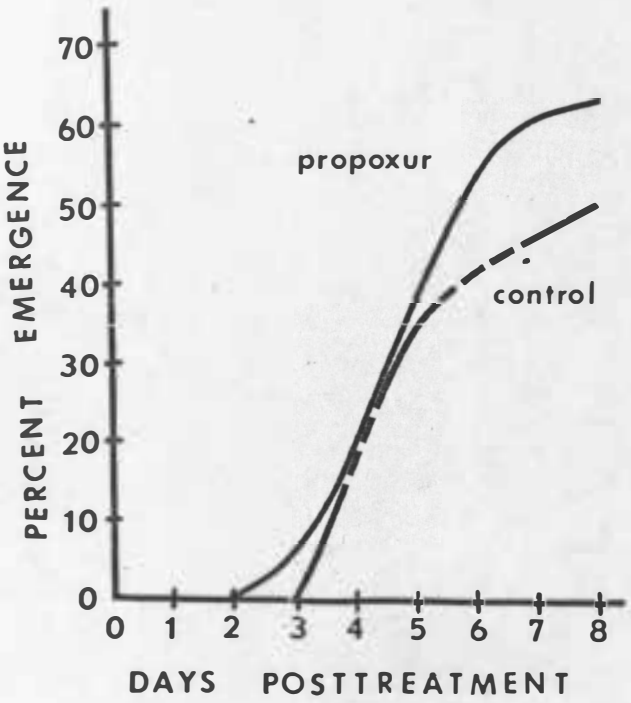
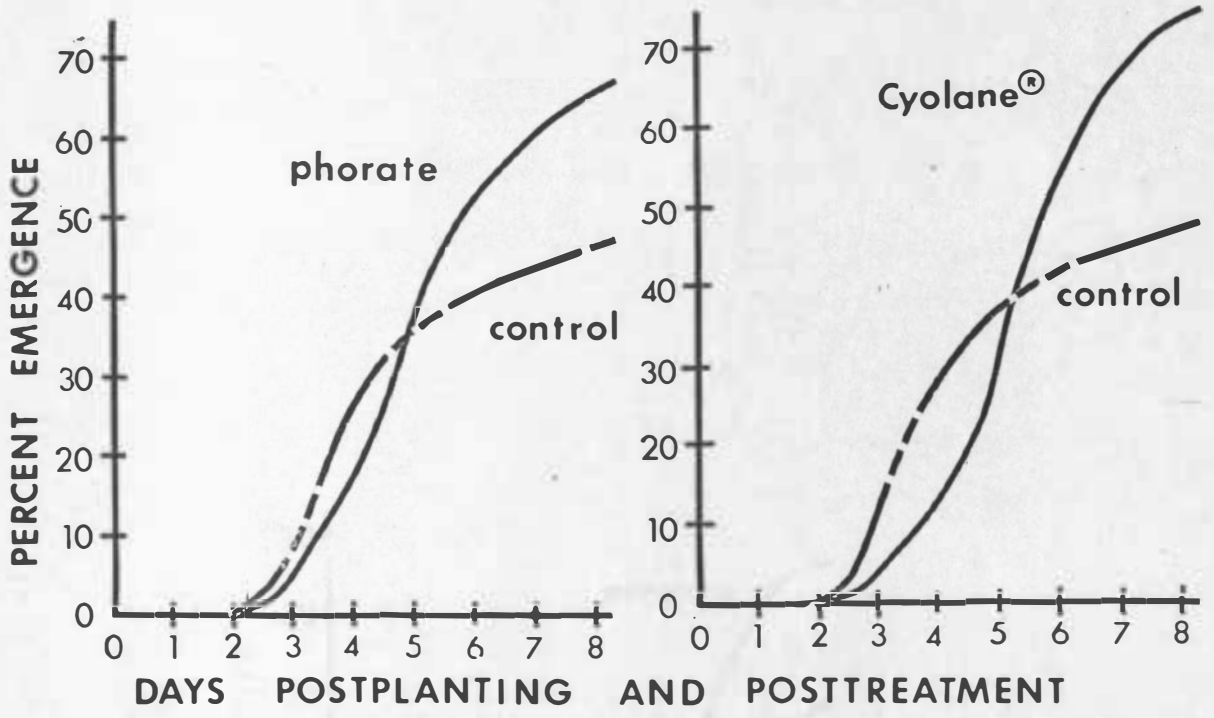
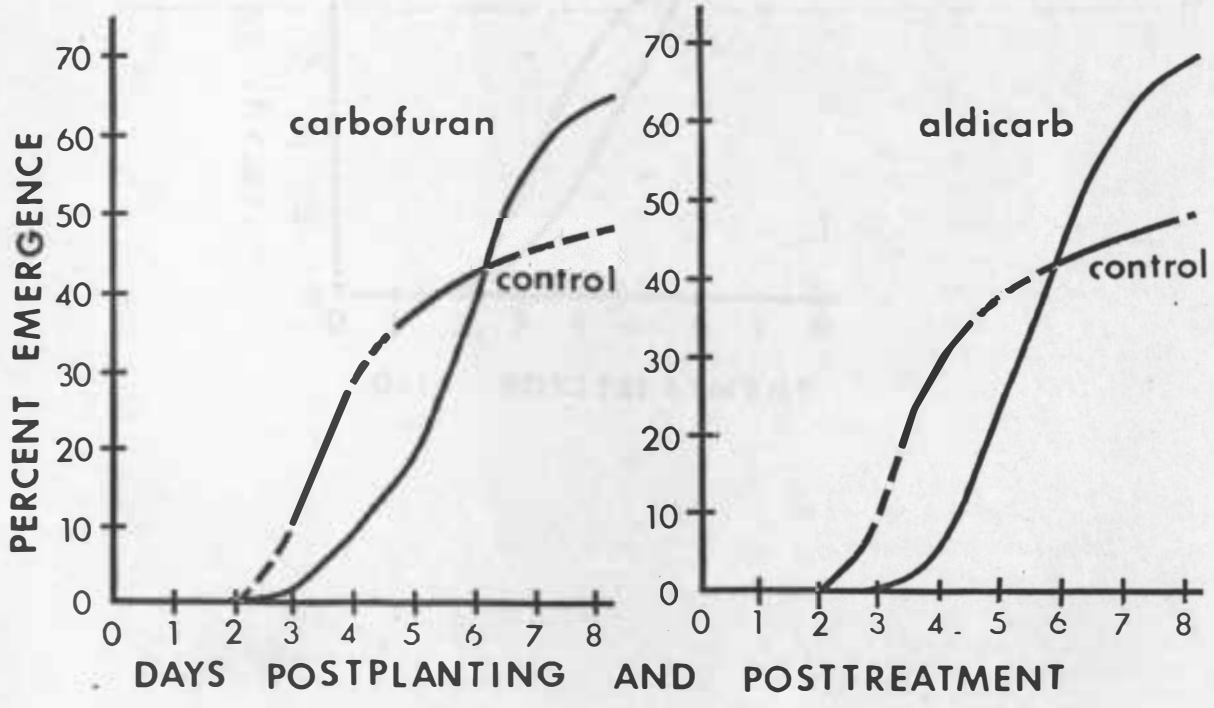
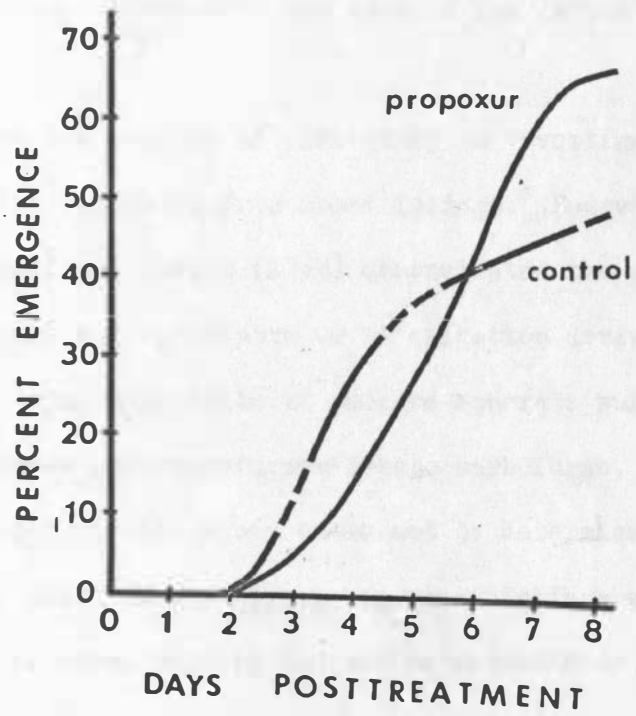

70
60
50
40
30
20
10
0
PERCENT EMERGENCE
propoxur
control
0 1 2 3 4 5 6 7 8
DAYS POSTTREATMENT

Figure 12.-Percent emergence of wheat grown in soil treated with insecticides at the rate of 11.20 kg/hectare.


PERCENT EMERGENCE
phorate
control
Cyolane®
control
DAYS POSTPLANTING AND POSTTREATMENT


PERCENT EMERGENCE
carbofuran
control
aldicarb
control
DAYS POSTPLANTING AND POSTTREATMENT


70
60
50
40
30
20
10
0
PERCENT EMERGENCE
propoxur
control
0 1 2 3 4 5 6 7 8
DAYS POSTTREATMENT

DISCUSSION AND CONCLUSIONS

Dosage-mortality data and bioassay data did not agree regarding the effectiveness of the materials tested as insecticides. Dosage-mortality data demonstrated that carbofuran and phorate (LD_{50} values of 0.105 ug/ul and 1.750 ug/ul respectively) were the most toxic to cutworm larvae. Data obtained from the army cutworm bioassay indicated that Cyolane caused the highest mortalities. One-half inch larvae were used in all tests. Therefore, the size of the larvae did not constitute a variable.

It was not the purpose of this study to investigate metabolites of each insecticide recovered from wheat foliage. However, Metcalf (1966, 1968) and Metcalf and Fukuto (1957) demonstrated the major metabolites of phorate, aldicarb and carbofuran to be oxidation derivatives of the parent compound; the major metabolite of phorate→phorate sulfoxide, aldicarb→ aldicarb sulfoxide and carbofuran→3-keto carbofuran. The carbamate insecticides used in this study could not be determined by the methods used. Phorate and Cyolane, per se, in wheat foliage were successfully determined by electron capture GLC and no metabolites of phorate and Cyolane were analyzed.

Since residues of phorate and Cyolane, per se, were present in wheat foliage 2 and 3-weeks posttreatment, surely, oxidative products were also present. The wheat plants exhibited high systemic capabilities for phorate and Cyolane, Cyolane more so than phorate. Cyolane was probably effective as a systemic in the wheat plant because of the high amounts of Cyolane residues in the plant which resulted in the high mortalities observed from the bioassay.

The carbamates tested in the dosage-mortality test gave high LD_{50} values except carbofuran. Since high LD_{50} values were noted using aldicarb and propoxur, low bioassay mortalities were expected and confirmed by the bioassay data. Carbofuran was found to be quite toxic when applied topically but the bioassay data indicated no appreciable mortality to cutworm larvae. Carbofuran and phorate, therefore, can not be considered as effective systemics in wheat based on the results indicated by the present data.

Metcalf (1966) reported that aldicarb applied to soil promoted increased yield of cotton plants from a few hundred to 900 pounds of seed cotton per acre. He concluded that increased yields were due not only to the suppression of pests attacking cotton plants but that aldicarb also directly affected the increased yields of seed cotton. It was demonstrated in the present study that phorate and propoxur treated soil increased germination and, hence, emergence of wheat plants was greater than in the control.

SUMMARY

Studies were made to determine the extent of systemic capability of various insecticides in winter wheat and the systemic insecticidal effect on larvae of the army cutworm. Data on determination of LD_{50} values, bioassay, residues and germination of wheat plants were obtained.

The insecticides phorate, Cyolane, aldicarb, carbofuran and propoxur were used in this study. Insecticides were applied to the soil at 1.12, 2.24, 5.60 and 11.2 Kg/hectare at a depth of ½ inch. Wheat seeds were planted and grown under greenhouse conditions. Two weeks posttreatment, wheat foliage was sampled, extracted and residues of phorate and Cyolane were determined by electron capture GLC but residues of aldicarb, carbofuran and propoxur were not detectable by the electron capture gas-liquid chromatographic methods used.

Cyolane was the only effective systemic insecticide in wheat of the insecticides tested. Mortality of army cutworm larvae was slight for all insecticides tested except Cyolane.

Germination of wheat seed was stimulated upon application of phorate and propoxur to the soil. Cyolane, aldicarb and carbofuran affected no significant increased germination of wheat seeds over that of the control.

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